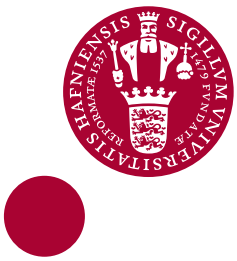

Applied Hologenomics

Feasibility and Potential in Aquaculture

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Applied Hologenomics: Feasibility and Potential in Aquaculture

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Highlights

- An animal host's genotype and gut microbiota both play significant roles in shaping key phenotypes of aquacultural relevance including growth metabolism and immune functions.
- Traditional approaches to improve production have relied on selecting for direct genotype-phenotype correlations or attempted to directly modulate gut microbiome communities.
- The hologenome theory argues that the genomes of host organisms and their associated microbial communities are subjected to biological interactions and cannot be viewed independently.

- The gut microbiota can be viewed as a collection of genotypes contributing to holobiont phenotypes and linked to the host genotype, and any attempts to modify the gut microbiota can only be successful in the context of the host genotype 'environment'.
- A hologenomic approach to aquaculture has potential to improve growth, health and sustainable production.

Abstract

Aquaculture will play an essential role for feeding a growing human population, yet certain biological challenges impede sustainable growth in production. Emerging evidence across all areas of life has revealed the importance of the intimate biological interactions between animals and their associated gut microbiota. Based on challenges in aquaculture, we leverage current knowledge in molecular biology and host-microbiota interactions to propose an applied holo-omic framework that integrates molecular data including genomes, transcriptomes, epigenomes, proteomes, and metabolomes for analyzing fish and their gut microbiota as interconnected and co-regulated systems. With an eye towards aquaculture, we discuss the feasibility and potentials of our holo-omic framework to improve growth, health, and sustainability in any area of food production, including livestock and agriculture.

Keywords: Aquaculture; Sustainable production; Hologenome; Holobiont; Holo-omic analysis

Aquaculture's critical role in food production in the 21st century

With the global population fast approaching the nine billion mark, one of the key challenges of the 21st century is to secure sustainably increased food production [1]. Climate change is predicted to reduce the land area suitable for

terrestrial livestock production [2], while production of aquatic resources is expected to grow in response to this need. Aquaculture is already the fastest growing food industry, with almost half of all consumed fish currently being farmed [3]. Furthermore, global aquaculture production is predicted to grow by at least 5% per year in the foreseeable future [3, 4]. Aquaculture's unprecedented growth prompts an urgent need for its optimization, through scientific solutions to improve fish growth and health, while minimizing the environmental burden [3]. Thus, ideal solutions to these challenges will not only improve feed conversion efficiency, but also simultaneously reduce both eutrophication from feed waste and the transfer of diseases to wild fish stocks. In this article, we discuss emerging opportunities that come from technological developments and decreasing costs in the field of molecular biology.

From genomes and metagenomes to hologenomes

Considerable efforts have already been directed towards developing biotechnological solutions to address aquaculture's most urgent challenges. In particular, the application of molecular tools in aquaculture has undergone a revolution over the last decade due to the rapid development of DNA sequencing technologies [5]. So far, such tools have mainly been used to explore how phenotypic traits related to growth or health are associated with variation in either (i) the fish **genome** (see Glossary) or, more recently, (ii) its **gut microbiota** (Table 1). However**,** hosts are not isolated from their intestinal (symbiotic and commensal) microorganisms, but rather can be interconnected and co-regulated systems that condition host phenotypes (Figure 1, Key Figure).

We believe that the **hologenome** concept [6-8] can be exploited in light of this observation. Specifically, we advocate that any attempts to understand how fish (or indeed any complex eukaryotic host species) adapt and acclimate to changes – whether it be the local environments, the diet, or disease challenges – must consider the host genome and the intestinal microorganisms' **metagenome** (hereafter simply metagenome) together: the hologenome [9]. In this way, intestinal microorganisms are not considered passive passengers, but rather active crew, who can shape phenotype and behavior of the host organism. This has powerful implications for, not only fundamental processes within evolutionary biology (e.g. how species adapt, compete, speciate, become domesticated, or go extinct), but also applied questions within the agrisciences, including aquaculture (Boxes 1-2).

Examples already exist that clearly illustrate how hosts and their microbiota are interconnected. The presence of particular microbes within the fish gut provides essential functions for the host by stimulating fatty-acid metabolism [10, 11], or epistatic control of host immune genes [12, 13]. Host genotypes are also known to shape the microbial composition – and hence likely the function – of the microbiota, as evidenced by distinct microbial community profiles between sexes [14] and among individual genotypes within a population [15-17]. However, despite these findings, little is known about the mechanisms governing the interactions between host genomes and those of their resident microbes, and how these mechanisms affect host phenotype.

Studies on fish microbiota have largely been limited to descriptive characterizations of taxonomic compositions, thus revealing little insights into

the metabolic functions provided by the gut microbiota [18, 19]. In light of this, we advocate the need to integrate molecular data derived from both host and symbiotic microorganisms into an integrative multi-omic framework, which we coin the **holo-omic framework**. This framework should enable examination of biological interactions between the genome and the metagenome at multiple -omic levels, ultimately providing information that can be used to improve production practices. In this article, we argue that taking such approaches represents a critical step towards achieving more sustainable growth of the aquaculture sector.

The Holo-omic Framework

We advocate that the adoption of holo-omic approaches would overcome the limited functional insights of current analytical strategies, by simultaneously considering the **holobiont** (host and its associated microbiota) at multiple molecular levels. This involves deciphering interactions between not only the host's genome, but also its **epigenome** and **transcriptome,** and its microbial metagenome and **metatranscriptome**. Studies would ideally also incorporate analyses of the associated **proteomes** and **metabolomes**, and **metaproteomes** and **metametabolomes**, to fully recover the functional pathways controlling the host phenotype (Figure 2). Successful integration of such data into a holo-omic framework will reveal mechanisms such as how host genomes regulate the composition of the microbial community, or, conversely, how certain microbes interact to control host gene expression patterns. The full holo-omic approach provides the potential to move from simply knowing *what microbes are within the host* to understanding *how do hosts and microbes interact*. This information

will open the door towards new applications for actively promoting beneficial functions provided by the microbiota associated with the host fish.

While multiple authors have praised the benefits of **multi-omic** approaches, very few have suggested strategies to integrate them [but see 20]. Here, we propose a progressive and flexible workflow that integrates the above mentioned omics datasets, which can be modulated depending on the research question and financial capacity. The workflow aims to identify the hologenomic interactions that produce phenotypic variation. It is primarily devised to compare cohorts under different treatments or environmental conditions, but it could be implemented in virtually any experimental design. The workflow is divided into two analytical phases (Figure 2). The first phase is based on association analyses that aim to identify molecular variants associated with phenotypic traits (e.g. growth or health related traits). The second phase is based on analyses of the functional interactions among the omics levels that shape such host-microbe-phenotype associations.

Step 1: Association phase

We propose to first perform association analyses on both host genomes and intestinal metagenomes, in order to identify associations between host phenotypes and genomic or metagenomic variants. **Genome-wide association studies (GWAS)** of host genomes have been common practice since the development of high throughput genotyping techniques. GWAS has been principally used to detect associations between diseases and single nucleotide polymorphisms (SNP) in the human genome [21], but GWAS also served to identify a mutation with a major effect on age of maturity in Atlantic salmon [22].

Metagenome-wide association studies (MGWAS) [23] are more recent and less commonly used, and yet to be implemented in aquaculture or agrisciences in general. A MGWAS approach connects host phenotypic variation to specific microbial genes, e.g. based on relative gene abundance within a host's metagenome [23]. These association-based analyses can be extended to the epigenomic and transcriptomic domains, by using genome-wide methylation or gene expression patterns [22, 24].

The association phase identifies candidate variants that might explain variation in growth, health, or any other phenotypic trait of relevance to aquaculture. Results from this phase provide important first insights, revealing whether phenotypic changes correlate with genomic and/or metagenomic variation. The link between genomic and metagenomic variants with host phenotype is not however direct, but probably subjected to a myriad of molecular interactions between hosts and symbiotic microorganisms (Figure 1B). For example, intestinal metagenomic variants might condition host gene expression [12, 25], or inversely, the host genotype and host metabolome might shape metagenomic variants [26]. Thus, deciphering the molecular underpinning of phenotypic variation will require more detailed analyses of the molecular networks that link genomes and metagenomes with host phenotype. Results from this association phase thus narrow a focus for designing subsequent analyses in the following *interaction phase*.

Step 2: Interaction phase

The second phase aims to disentangle hologenomic interactions that occur between hosts and gut microorganisms at different molecular levels. In

contrast to the first phase, associations are established among different -omes (e.g. host transcriptome and symbiotic metagenome) rather than between -omes and host phenotypes (e.g. symbiotic metagenome and host growth). For instance, GWAS can be used to identify associations between host genes and taxonomic and functional gut microbiota profiles [27, 28], which allows identification of host genes that might control the composition and function of the intestinal microbial communities [27]. Similarly, methylation-wide association studies can be performed to detect putative links between the host's epigenome and its microbial community [24].

Composition of the gut microbiota often changes throughout the life cycle of the host in response to environmental factors; therefore associations between variation in host gene expression (transcriptome) and microbial taxonomic or functional profiles might provide further insights into how host gene expression affects its associated microbiota. When the metatranscriptome is also analysed, it should be possible to assess whether host and microbial gene expression are compensatory (i.e. genes with similar functions are overexpressed in one system and underexpressed in the other), complementary (different genes within the same metabolic pathway are expressed in the two systems) or antagonistic (one system responds with defensive mechanisms to the gene expression of the other). However, unveiling the direction of the causal relationship might be difficult when studying host transcriptomes and metatranscriptomes together, as the effect might be controlled by reciprocal feedback mechanisms [29, 30].

Expansion of datasets to also include (meta)proteomes and (meta)metabolomes would help complete holo-omic analyses, by improving

resolution, and thus, conferring the potential to detect possible causal mechanisms. It is important to note that unbiased profiling of all peptides and metabolites present in complex intestinal or faecal samples is complicated, and only a fraction of the total pool of proteomic and metabolomic elements is usually captured [20]. The exact protocol to be used should therefore be chosen on a case-specific basis, to ensure that relevant proteins and metabolites are characterized.

Finally, proper implementation of the holo-omic framework will require i) high-quality samples preserved using sample specific methods (e.g. ethanol for DNA, RNAlater for RNA), ii) generation of deep high quality molecular data using different technologies (e.g. high throughput sequencing and mass spectrometry), and iii) the application of complex multivariate statistical treatments designed to match the question at hand.

Example applications of the Holo-omic Framework

As the economic cost of molecular data generation is decreasing, we propose that adoption of holo-omic approaches will become more relevant to the aquaculture sector – or indeed, the production of any livestock. Here, we discuss how the holo-omic framework presented above can be used to guide research towards the development of novel solutions for improving aquaculture production. It is important to note that research should be driven by a concrete question, which might require only some parts of the holo-omic framework (Figure 2), thus, prohibitively high costs can be avoided. The research question can then be addressed by adding holo-omic thinking to a standard formula for applied research exemplified in the following workflow:

1. Identify which of the omics levels are related to the trait in question; e.g. specific gut microbial species or host genotype.

2. Apply insights to develop: i) diagnostic biomarkers for health and growth traits, or ii) feed additives that boost healthy gut microbiota.

3. Test the industrial feasibility of such new solutions in carefully designed follow up studies.

4. Implement the application of new holo-omic solutions in aquaculture.

Below we contextualize our proposed framework by discussing its relevance in light of current challenges in aquaculture that relate to the optimization of growth and health traits in farmed fish. We provide concrete examples in Boxes 1 and 2 that illustrate areas where the holo-omic approach can improve sustainable production.

Growth

The metabolic conversion of feed into fish biomass is a pivotal process in fish production. Despite a long-standing appreciation of the gut microbiota's role in feed conversion, we have only recently been able to directly study the functions of gut microbes in fish [31]. It is without doubt that uncovering the metabolic functions of gut-associated microbiota will help improve feed conversion. Below, we focus on two current challenges where a holo-omic approach is likely to offer new solutions.

There is currently an increased global demand for fish meal derived from wild small pelagic fish [3]. Given these wild resources are limited, thus not sustainable in light of aquaculture's predicted growth, the industry is increasingly forced to consider alternative raw materials such as plant based oils and protein [32, 33]. However, since many aquaculture species are **piscivorous**, there may well be detrimental metabolic and health consequences when fish are fed a purely 'vegetarian' diet. It would therefore be relevant to combine genome and metabolome data with the metatranscriptome and metametabolome, to identify functions that lead to dysbiosis when piscivorous fish metabolize a plant-based diet. An ultimate solution could then include the use of microorganisms to pre-digest plant derived ingredients or directly prime gut microbiota to improve digestibility of plant based feed (Box 1).

Another important aspect of fish farming is controlling the fatty acid (FA) content in muscle tissue. In particular, long chain omega-3 FAs, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), contribute to healthy consumer products. It has been argued that omega-3 FAs from farmed fish may be healthier for humans than those from wild counterparts of the same species [34]. Fish feed companies currently invest considerable resources into the direct addition of FAs derived from expensive raw materials into their feeds, with the intent that the fish will absorb the added FA into muscle tissues [3]. While this approach helps provide fish products that are rich in healthy FAs, we hypothesize that more efficient alternatives involving the gut microbiota can be developed. One approach could be to combine omics data from both the host and its gut microbiota to identify microorganisms affecting FA content in the host's

muscle tissue. Future applications could include use of pre- or probiotics to stimulate growth of such microorganisms known to improve FA absorption (Box 1).

Health

Disease outbreaks, impaired health and potential transfer of diseases to wild fish stocks represent a major concern for many fish farms. Treatment of some diseases often requires the use of chemotherapeutics, which further burdens the environment. We argue that future solutions based on the holo-omic framework hold the potential to be both greener and more efficient in treating diseases.

Common diseases include enteric pathogens infecting fish through their digestive canal, in particular bacteria within the genus *Aeromonas* [35]. Such pathogen outbreaks reduce fish welfare, and often require stock treatment. Combatting pathogens through specific antibiotics that are naturally provided by gut bacteria could not only improve fish welfare, but concomitantly reduce the development of resistant pathogenic strains, minimize economic losses, and reduce the environmental burden from chemotherapeutic agents [36]. By integrating data on host genome, gut microbiota composition, and metaproteome, one would be able to identify natural gut microorganisms that limit colonization of pathogens. Ultimately, one may attempt to boost immune activity of these microorganisms through selective breeding for host genotypes associated with a strong microbial immune function (Box 2).

Extrinsic control of host gene expression provides another potential for combatting disease. Many vertebrate immune genes and their functions are well characterized in some model organisms, and it is becoming increasingly clear that intestinal gut microorganisms can control regulation of some host immune genes [25]. However, we know very little about the actual mechanisms by which these host-microbiota interactions occur. One study considered germ free juvenile zebrafish to show that inoculation of commensal gut microbes directly primed a transient inflammatory response of some immune genes; this hologenomic interaction led to increased resistance against infectious diseases [12]. Application of the holo-omic framework could be used to identify interactions among the host transcriptome and the metagenome, to identify microbial species associated with expression patterns of host immune genes and potentially antibiotic substances in the host proteome. Such knowledge could inspire new applications that boost growth of specific microorganisms known to beneficially interact with host immune responses (Box 2).

Concluding remarks and future perspectives

Hologenomic thinking is still in its infancy [8], and to our knowledge, it has yet to be integrated into applied research. Looking forward, we encourage the scientific community to increasingly apply hologenomic thinking, and promote collaboration among scientific, political, and industrial players if we are to fully realise the potential of the hologenome concept in aquaculture and other food sectors (see Outstanding Questions). We conclude by discussing some of the key efforts needed going forward.

Scientific infrastructure

Admittedly, in planning a new project applying the holo-omic approach outlined in Figure 2, the mere cost of generating the multi-omic data may be out of scope for many research budgets, particularly so in non-model and less developed aquaculture species. Early efforts are thus likely to be carried out on species from the carp, salmon, tilapia, and catfish families with the most molecular resources available [3]. Findings from these species will then result in the development of optimised protocols, such as bioinformatics tools to perform integrated analyses of holo-omic data sets, which will allow more cost-effective analyses in other species [see e.g. 20].

Also, each new project will generate invaluable molecular resources and continue to improve reference databases for common fish gut microorganisms. Further, the accumulation of new species-specific reference metagenomes and microbial reference databases will improve annotation efforts and the overall information that can be gained from a holo-omic data set. Lastly, the per unit cost for generating sequence data is expected to continuously drop, thus rendering such techniques more accessible to most research budgets [37].

Motivate joint efforts between industry and academia

Efforts to better align motivation and research interests between commercial and academic partners are needed. First, industrial partners should, to a larger degree, be included in scientific committees on applied research projects to ensure mutual interests are aligned among stakeholders from the industry, academic research, as well as end consumers. Second, the novel approach of open innovation platforms should be considered. Innovation platforms provide an open forum where companies and academics are working

together to solve societal challenges [38]. Many globally acting companies are already investing in these platforms to support novel ideas with a high potential for solving a concrete challenge. Indeed, a recent review highlighted the high potential of open innovation in the aquaculture sector [39]. Applied hologenomics has the potential to receive the right attention and consequently receive financial support to address current challenges in aquaculture.

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Outstanding Questions Box

- To what degree are the gut microbiota and its functions controlled by host genotype, and thus predictably recurrent between generations of broodstock fish?

- Is it possible to use hologenomic markers to screen broodstock fish to select for traits such as host-microbiota interactions and improve fish health?

- How plastic is the established gut microbiota in host fish, and to what degree can it be modulated through feed additives?

- How do we reduce the currently high costs of performing holo-omic analyses to a level that is financially feasible for high throughput analyses in species with limited financial resources?

- How can the aquaculture industry be motivated to engage in the long-term research programs that are needed to fully realize the potential of the holo-omic approach?

Box 1. Improving feed utilization and growth

Example 1 – Replacing fish meal

Rhodes and colleagues [53] evaluated the effects of substituting fish oil with either flaxseed oil or corn oil on growth and health in sablefish (*Anoplopoma fimbria*). Their results were concerning - fish reared on the alternative plant based diets suffered reduced growth, increased liver damage, and reduced gut microbiota diversity [53]. We argue that one solution may be hidden within the gut microbiota, and propose a holo-omic approach to reveal the underlying mechanisms of this metabolic dysbiosis and potential solutions.

Holo-omic approach

1) Combine genome and metabolome data with the metatranscriptome and metametabolome, to identify metabolic functions that lead to dysbiosis in piscivorous fish feeding on a plant-based diet (Figure IA).

2) Study herbivorous species to identify microorganisms and enzymes that are involved in the metabolism of plant components.

3) Test how the plant metabolizing microorganisms thrive in piscivorous fish and how they may be affected by the host genotype.

Holo-omic solutions

1) Design tailor-made feed packages that stimulate growth of plant metabolizing gut microbiota while matching the host genotype background.

2) Use enzymes to pre-digest plant-based ingredients into a state that is more easily metabolized by the natural microbiota of piscivorous fish.

3) Use CRISPR-Cas9 to modify naturally occurring gut microorganisms' ability to metabolize plant material by mutating genes to mimic those in metagenomes of herbivorous species.

Example 2 – Controlling fatty acid composition

Yazawa, et al. [55] detected a positive correlation between the eicosapentaenoic acid (EPA) content of several marine shellfish and fish species, and the presence of EPA synthesizing gut bacteria. More recent studies have revealed a direct diet-dependent role on microbiota mediated FA absorption in the intestines of zebrafish (*Danio rerio*) [10, 11]. We argue that the holo-omic framework can help develop solutions to actively modulate metabolic host-microbe interactions in the FA pathway and improve feed conversion.

Holo-omic approach

1) Associate host FA profile with variation in genome, metagenome, metatranscriptome, metaproteome, and metametabolome to identify bacterial genes that mediate FA absorption (Figure IB).

2) Consider the metaproteome and metametabolome to decipher molecular pathways by which intestinal bacteria interact with host epithelial tissue to mediate FA absorption.

Holo-omic solution

1) Design feed additives (e.g. pre- or probiotics) that stimulate growth of bacteria known to improve FA absorption.

2) Match FA based feed ingredients to the needs of the natural gut microbiota.

Box 2. Improving health and disease resiliency

Example 1 – Treatment against intestinal pathogens

An interesting idea relates to the use of intestinal gut microorganisms as a natural source of targeted antibiotics for fighting external pathogens. Indeed, the probiotic species *Carnobacterium maltaromaticum* and *C. divergens* have been shown to inhibit growth of pathogenic *Aeromonas*, *Streptococcus*, and *Vibrio* strains [56]. Again, the holo-omic framework offers a new way to identify and utilize similar, but yet unknown, interactions to boost immune function of the holobiont host while reducing the need for chemotherapeutic agents.

Holo-omic approach

1) Combine data on host genome, gut microbiota composition, and metaproteome to characterize commensal microorganisms that provide antibiotic functions (Figure IIA).

2) Assess how the community structure and immune activity of the gut microbiota depend on host genotype.

Holo-omic solution

1) Develop pre- and probiotics to boost growth of particular bacteria shown to provide antibiotic activities.

2) Perform selective breeding (or even genome editing) targeting host genotypes associated with high abundance of gut bacteria that actively fight against enteric pathogens.

Example 2 – Microbial regulation of host immune genes

It has been shown that active feeding of the probiotic *Pediococcus acidilactici* to Nile tilapia (*Oreochromis niloticus*) boosted the immune capacity of the host fish [25]; after only six weeks the authors observed an increased abundance of *Pediococcus acidilactici* strains in the gut. Interestingly, the probiotic was further associated with elevated expression of intraepithelial leucocytes and the pro-inflammatory cytokine tumour necrosis factor-a genes in the Nile tilapia host tissue [25]. This example highlights the potential of applying the holo-omic framework to identify, and eventually modulate, similar host-microbiota interactions to improve disease resilience.

Holo-omic approach

1) Identify holo-omic interactions among host genome, transcriptome and the metagenome and gut microbiota community to identify microbial effects on expression of immune genes - including antibiotic substances in the host proteome - and how this depends on host genotype (Figure IIB).

Holo-omic solution

1) Re-think treatment protocols that broadly kill gut microorganisms (e.g. standard broad-spectrum antibiotics), to design new treatment protocols including live microorganisms known to positively influence expression of host immune genes.

2) Develop probiotic packages designed to target a specific host genotypic background as different **gene variants** are expected to respond differently to the same microbial stimuli.

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Glossary

Epigenome: the modification of DNA (or proteins associated with DNA) of an organism that modifies its gene expression patterns without altering the DNA sequences.

Genome: the complete set of an organism's genetic material.

Gut microbiota: a community of microorganisms that live in the digestive tracts of a host organism.

Genome-wide association study (GWAS): an examination of a genome-wide set of genetic variations associated with a trait of interest.

Holobiont: a host and all its associated microbes.

Hologenome: all genomes of a holobiont.

Holo-omic framework: analytical approach to integrate multiple omics data at different molecular levels from a host and its associated microbiota.

Metabolome: the complete set of metabolites within an organism or system.

Metagenome: combined genetic material present in an environmental sample (e.g. gut content), consisting of the genomes of many individual organisms.

Metametabolomes: the complete set of metabolites synthesised by a community of interacting organisms or species.

Metaproteome: the complete set of proteins/peptides synthesised by a community of interacting organisms or species.

Metatranscriptome: the complete set of messenger RNA synthesised by a community of interacting organisms or species.

Metagenome-wide association study (MGWAS): an examination of a metagenome-wide set of genetic variants associated with a trait of interest.

Multi-omics: an integrative analysis where the data consist of multiple omes such as genome, epigenome, transcriptome, proteome, metagenome and metabolome.

Piscivorous: feeding on fish

Proteome: a complete set of proteins in a sample that is synthesised by the host organism.

Transcriptome: a complete set of messenger RNA in a sample that is expressed by the host organism.

Figure legends

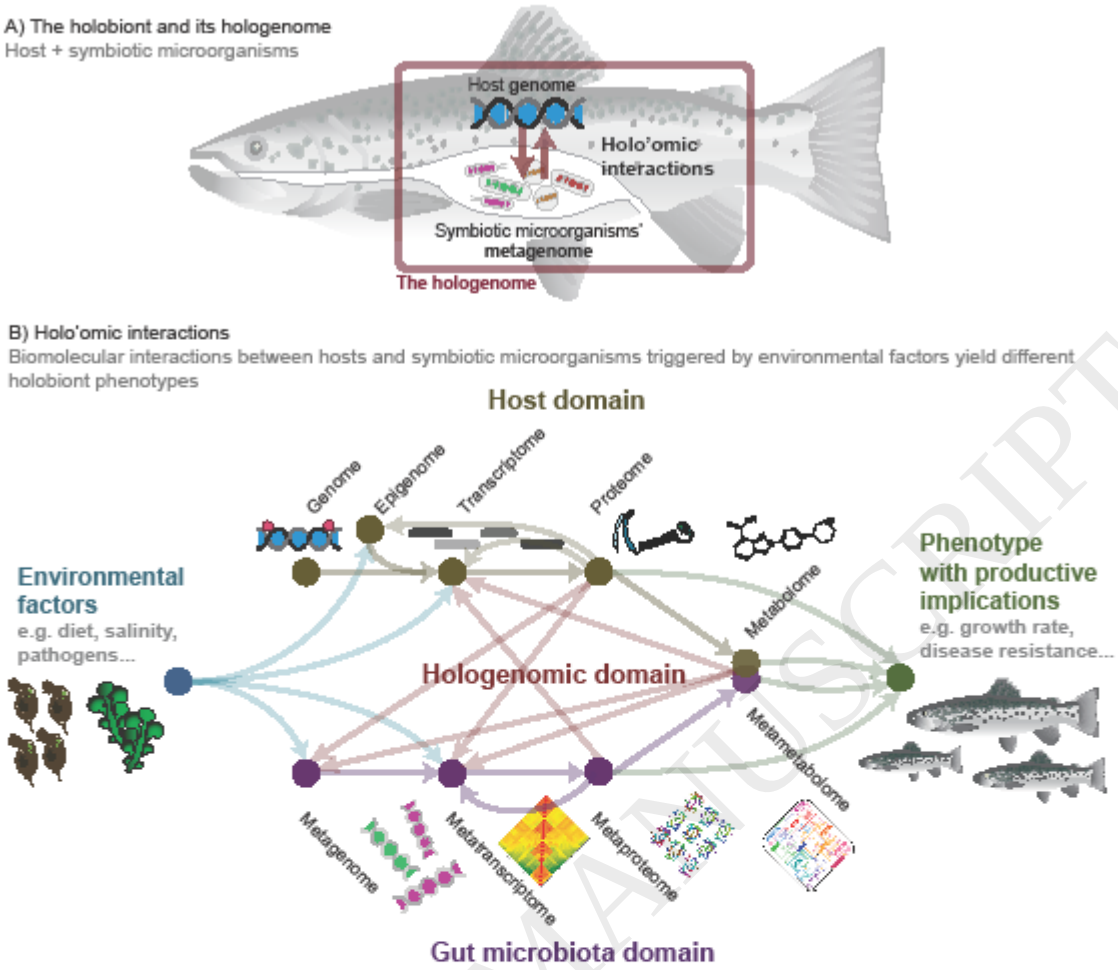
Figure 1, Key Figure. Conceptual framework of the holobiont and the hologenome (A), and the holo-omic interactions between the host and its gut microbiota (B). Arrows indicate the directionality of the effect. Blue arrows indicate environmental effects, which might affect metagenomic composition, gene expression of both host and their intestinal microorganisms, and also introduce epigenomic variation. Brown arrows show molecular interactions within the host domain, while purple arrows show molecular interactions within the gut microbiota domain. Red arrows highlight holo-omic interactions, i.e. potential reciprocal effects between host and their intestinal microorganisms at different omics levels. Finally, green arrows link all those interactions with host phenotypes. Note the overlapping circles of the host metabolome and the microbiota metametabolome, which indicates that often metabolites cannot be assigned either to the host or the microbiota, but are the result of the combination of both domains.

Figure 2. The two-step analytical procedure of the proposed holo-omic framework. In Step 1, the 'Association phase', genomic and metagenomic data are generated from host and microbial DNA extracts, and the genomic or metagenomic variants are linked to host phenotypes through genome-wide (GWAS) and metagenome-wide (MGWAS) association analyses, respectively. In Step 2, the 'Interaction phase', other omic data are generated to characterize interactions among the different host and microbial omic levels, and to improve our understanding of how genomes and metagenomes interact to produce different phenotypes. Pink arrows indicate host effects upon microorganisms,

while orange arrows indicate the opposite action. Thick arrows indicate the dominant interactions in each direction: i) how host genotype affects microbial functional profiles, and ii) how microbial functions affect host gene expression.

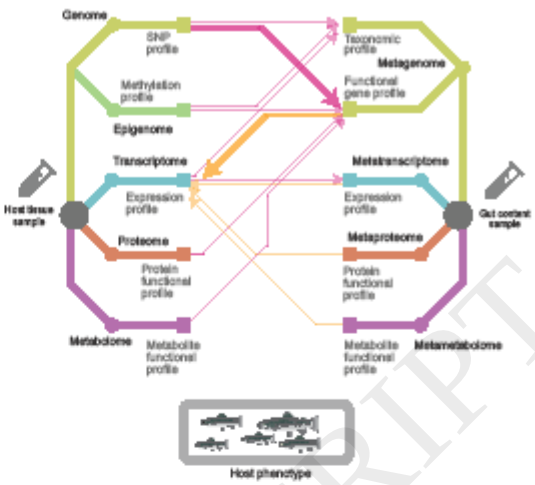
Figure I. Illustration of suggested holo-omic approaches for tackling the challenges discussed in Box 1. Examples include replacement of fish meal (A) and controlling fatty acid metabolism (B).

Figure II. Illustration of suggested holo-omic approaches for tackling the challenges discussed in Box 2. Examples include treatment against intestinal parasites (A) and microbial regulation of host immune gene expression (B).



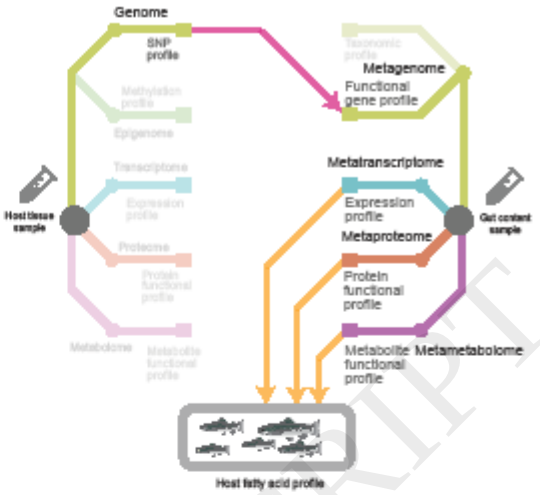
A) **Step 1**: Association phase

B) **Step 2**: Interaction phase



A) Example 1. **Replacing fish meal**

B) Example 2. **Controlling fatty acid composition**



A) Example 1. **Treatment against intestinal parasites**

B) Example 2. **Microbial regulation of host immune genes**

Table 1. Example studies linking variation in either host genome or host microbiome, with phenotypic traits of relevance to aquaculture production.

Molecular level	**Species**	**Research question**	**Key findings**	**Refs**
Host genomic domain	Atlantic salmon (*Salmo salar*)	Disease resistance	A mutation in the Cdh1 gene provides resistance to IPN virus	[40, 41]
		Age at sexual maturity	Vestigial-like family member 3 gene (VGLL3) promotes sex-specific maturity	[22]
		Growth and fillet traits	Two SNPs associated with growth and fillet traits	[42]
	Golden Pompano (*Trachinotus blochii*)	Growth	Polymorphisms within the leptin-a gene associated with growth traits	[43]
	Bighead carp (*Hypophthalmichehys nobilis*)	Growth	A SNP in the TP53BP2 gene associated with growth traits	[44]
	Catfish (inter-specific hybrid)	Disease resistance	The nck1 gene may provide resistance to the bacterial pathogen *Edwardsiella ictaluri*	[45]
	Asian seabass (*Lates calcarifer*)	Disease resistance	The rtp3 gene may provide disease resistance to nervous necrosis virus	[46]
Host gut microbiota domain	Atlantic salmon (*Salmo salar*)	Effect of diet on gut microbiome composition	Diet content affects gut microbiome composition	[47, 48]
	Rainbow trout (*Oncorhynchus mykiss*)	Effect of diet on gut microbiome composition	Diet content affects gut microbiome composition	[49]

		Functional description of gut microbiome	Identification of microbial genes likely involved in metabolism	[50]
	Nile tilapia (*Oreochromis niloticus*)	Effect of dietary supplements	Enhanced growth without changes in microbiome composition	[51]
		Effect of probiotics on immunological response	Probiotics can stimulate immune-responsiveness	[25]
	Fine flounder (*Paralichthys adspersus*)	Microbiome comparison of reared vs. wild specimens	Microbiome composition varies between reared and wild specimens	[52]
	Sablefish (*Anoplopoma fimbria*)	Effect of diet on gut microbiome composition	Diet content affects gut microbiome composition	[53]
	Turbot (*Scopthalmus maximus*)	Metagenome profiling of gut microbiome	Functional description of metagenome	[54]